OPINION PAPER

M. Susan Haas · Susan J. Brown
Richard W. Beeman

Pondering the procephalon: the segmental origin of the labrum

Abstract With accumulating evidence for the appendicular nature of the labrum, the question of its actual segmental origin remains. Two existing insect head segmentation models, the linear and S-models, are reviewed, and a new model introduced. The L-/Bent-Y model proposes that the labrum is a fusion of the appendage endites of the intercalary segment and that the stomodeum is tightly integrated into this segment. This model appears to explain a wider variety of insect head segmentation phenomena. Embryological, histological, neurological and molecular evidence supporting the new model is reviewed.

Keywords Insect head · Intercalary · Labrum · Stomodeum · Segmentation

Introduction

The accumulating evidence supporting the appendicular nature of the labrum (Cohen and Jürgens 1989; Haas et al. in press) reopens the question of its segmental origin. In insects it is an apical structure and has been variously characterized as (1) a simple cuticular flap of either the non-segmental acron or of the first segment, or (2) an appendage of either the anterior-most segment or of the intercalary segment (Matsuda 1965; Rempel 1975).

If appendicular, then the labrum cannot be a feature of an acron, which by definition, has no appendages (Snodgrass 1928). As a segmental structure, the labrum has puzzling characteristics that suggest it is an incomplete segment. In most insects and in crustaceans it lacks *engrailed (en)* expression (Patel et al. 1989; Schmidt-Ott et al. 1994; Scholtz 1995). In beetles, the labrum displays incomplete histological characteristics during development (Ullmann 1964; also see below). In *Drosophila,* ectopic expression of *Antennapedia (Antp)* does not induce a homeotic transformation of labrum to a thoracic identity (Lindsley and Zimm 1992; Rogers and Kaufman 1997) as it does in most other segments.

The intercalary segment, though also atypical, is now considered a true segment or segmental remnant, based on the presence of a neuromere (the tritocerebrum), embryonic coelomic sacs, and the expression of both *en* and *wingless (wg)*. This segment's unusual features include its incomplete histological characteristics during development (Ullmann 1964), and its lack of obvious appendages during either embryogenesis or adulthood. Transient intercalary appendage rudiments have been found in embryos of several species (Ando and Okada 1958; Tamarelle 1984; Fleig and Sander 1986), but they have not been characterized beyond documenting their brief appearance. The absence of *Distal-less (Dll)* gene expression in the insect intercalary segment (Cohen 1990) is consistent with the lack of distal (telopodite) appendage development. In crustaceans, the orthologous tritocerebral segment does bear a pair of *Dll*-expressing appendages, namely the second antennae (Panganiban et al. 1995**;** Popadic et al. 1998).

Here, we review a diverse body of evidence that supports the view that the labrum and the intercalary segment are actually components of the same segment.

Arguments for an intercalary segment origin of the labrum

Butt (1960) proposed that the labrum is a fusion of the appendages of the intercalary segment. Contesting this view, Matsuda (1965) pointed out that the existence of such a labro-intercalary segment would mean that "this segment would carry two pairs of appendages" – a complete pair from the labrum and another complete pair

M.S. Haas · R.W. Beeman (✉)
USDA, ARS, Grain Marketing and Production Research Center,
Manhattan, KS 66502, USA
e-mail: beeman@usgmrl.ksu.edu

S.J. Brown
Division of Biology, Ackert Hall, Kansas State University,
Manhattan, KS 66506, USA

from the intercalary segment. By definition, each insect segment has only one pair of appendages (Manton 1977; Kukalová-Peck 1992). However, if the labrum is composed of endites only (Haas et al. in press), and the transitory appendages of the intercalary segment represent the repressed palps (telopodites) only, then the sum of the labral and intercalary appendage parts equals one complete appendage pair rather than two. This configuration eliminates the basis of Matsuda's protest.

The recent discovery of *labial (lab)* gene expression in the proximal labrum as well as in the second antennal segment and appendages of the crustacean *Porcellio scaber* (Abzhanov and Kaufman 1999) also supports a second antennal (intercalary) origin of the labrum in arthropods. This is the first reported instance of *lab* expression in the arthropod labrum, and might represent either an ancestral expression pattern that has been lost in insects, or one uniquely derived in this crustacean lineage. It will be interesting to see what further studies in a wider range of arthropod classes will reveal.

Current technology might provide the means to clarify the fate of the intercalary appendages. In a study of grasshopper neurogenic development, Meier and Reichert (1990) were able to demonstrate, at the level of individual peripheral pioneer nerve cells, the serial homology of the transient abdominal limb buds with the thoracic legs. A similar study of the transient intercalary appendages, as observed in the Hymenoptera (Ando and Okada 1958), could provide insights into their origin and fate. Comparable studies of the labrum might help clarify its association with the intercalary segment and perhaps resolve part of the labral debate.

The presence of the stomodeum between labral and intercalary cuticular elements makes it difficult to visualize them as parts of a single segment. The neuromere of this segment, the tritocerebrum, is unique in that it, too, surrounds the stomodeum. In insects and crustaceans, the tritocerebral commissure remains on the posterior side of the stomodeum, while its ganglia are located anterior (or laterally in some crustaceans) to the stomodeum, in effect "bridging" this structure. Thus, accommodation of the developing stomodeum could be seen as similarly altering both the cuticular and neural development pattern of this proposed composite segment.

Another obstacle to this visualization is the commonly attributed segmental location of the stomodeum. Current head segmentation models (Fig. 1A,B) commonly place the developing stomodeum in the posterior ventral midline of the antennal segment (Rogers and Kaufman 1997; Fig. 1 D). However, this midline area has unique characteristics that suggest it might not represent antennal tissue. *en* gene expression is always discontinuous at the midline in the developing deutocerebral segment of all crustaceans and insects examined (Brown et al. 1994; Scholtz 1995; Rogers and Kaufman 1996), with the exception of the milkweed bug, suggesting that this segment might be incomplete at the midline. Similarly, the *lab* expression recently demonstrated in the circum-stomodeal area of the crustacean *P. scaber* (Abzhanov and
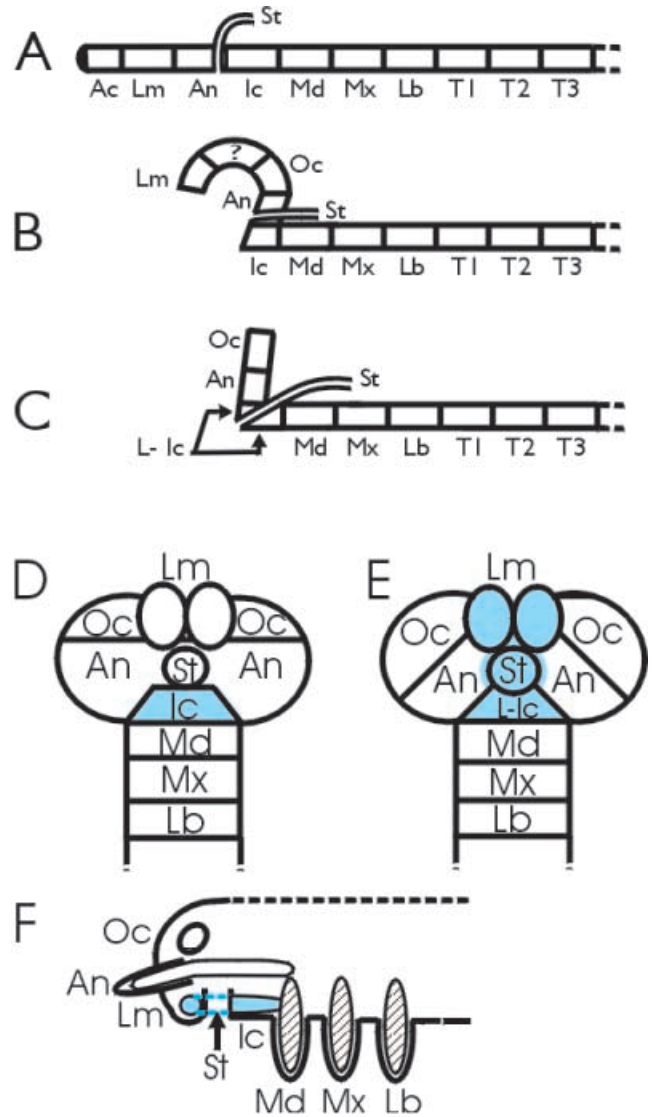

Fig. 1A–F Insect head segment sequence and orientation. Lateral view (**A–C,F**). Ventral view (**D,E**). **A** Linear model (after Rempel 1975). **B** S-model (after Schmidt-Ott and Technau 1992). **C** L-/Bent-Y model (this paper). **D** Linear and S-models. **E** L-/Bent-Y model. **F** *Tenebrio molitor* coelomic sacs, lateral view (interpreted from Ullmann 1964).*?* Unknown, *Ac* acron, *An* antennal, *Ic* intercalary, *L-Ic* labro-intercalary, *Lb* labial, *Md* mandibular, *Mx* maxillary, *Oc* ocular, *St* stomodeum, *T1-T3* thoracic segments 1–3, *blue color* intercalary or labro-intercalary surface (**D,E**), or labro-intercalary coelomic sac (**F**)

Kaufman 1999), if not an isolated example, also brings into question the identity of this midline area.

L-/Bent-Y model of insect head segmentation

We find that a revised model of insect head segmentation would better explain a number of anomalies, including those mentioned above, and propose the L-/Bent-Y model (Fig. 1C,E) which conforms to a wider variety of head capsule characteristics. This model envisions the pregnathal segment order (ocular, antennal, intercalary) as

proceeding in an anterio-lateral to midline sequence rather than a strictly anterior to posterior sequence. Additionally, the distal ends of this pregnathal sequence bend dorsally, giving the early embryo a "Bent-Y" appearance (seen as an L in lateral view). This model reinterprets the ventral midline surface, from the posterior border of the intercalary segment through the labrum, as labro-intercalary territory (Fig. 1E, blue). The antennal segment halves lie anterio-lateral to the labro-intercalary (L-Ic) and the ocular segment halves anterio-lateral to those of the antennal. Thus, in the L-/Bent-Y model, the stomodeum is seen as developing completely within the L-Ic segment, with which it becomes tightly integrated during subsequent development. Morphological, embryological and molecular support for this view is discussed below.

The pregnathal bend in head segmentation

Traditional views of insect head segmentation (Fig. 1A,B) consider the acron or the labrum to be the anterior-most structure. A different interpretation has been possible in studies of crustaceans due to the wider variety of head compositions and positions of the labrum within this class of arthropods. Both Beklemishev (1969) and Weygoldt (1979) note that in most crustaceans, a strictly linear view is valid. The apparent and actual anterior-to-posterior segmental sequence in these organisms is ocular, first antennal, second antennal and mandibular, with both mouth and labrum located on the ventral surface of the body. However, as Beklemishev notes, in higher crustaceans and in insects, "the mouth is directed forward and is almost terminal; the functionally-anterior end of the body is formed by the exterior margin of the labrum; both pairs of antennae (one pair in insects) are moved considerably to the dorsal side; and the eyes are dorsal. In the nerve chain the tritocerebrum is the ganglion farthest forward, whereas the deuto- and protocerebrum are bent dorsally and caudally, so that they lie above and slightly behind the tritocerebrum." Thus, higher crustaceans and insects are described as having a dorsalward bend in head segmentation, with the labrum and mouth at the bend.

Confirmation of such a dorsal bend in insect pregnathal segments has come from the analysis of expression patterns of the segment polarity genes *wg* and *en* in *Drosophila* by Schmidt-Ott and Technau (1992). They visualize an S-shaped bend of the pregnathal head segments, terminating anteriorly with the labrum (Fig. 1B). This dorsal bend, which agrees with Beklemishev's observation (above), also provides a possible explanation for an anomalous observation in Struhl's (1981) gynandromorph fate map study of the blastoderm primordia of the *Drosophila* head. He found that the eye-antennal primordium is rotated almost 180° from that of the gnathal primordium. Curiously, the labrum is unaffected. The linear model would predict that the labrum, presumed to be anterior to the ocular and antennal segments, should rotate with them and come to lie to their posterior. The resis-

tance of the labrum to this rotation could result in an S-shaped pregnathal segmental sequence. Alternatively, its rotational exclusion could indicate that the labrum is located posterior to, rather than anterior to, the ocular and antennal segments. This interpretation of Struhl's results is also consistent with a head segmentation model that assumes the labrum to be a fusion of intercalary appendages as proposed by Butt (1960). Such a configuration suggests an L-shaped bend, viewed laterally (Fig. 1C), with the ocular segment at the end, resulting in a six-segment head (ocular, antennal, labro-intercalary, mandibular, maxillary and labial). The labrum, situated at the bend, would appear to be the anterior-most structure of the head, though actually being the third segment – a visual deception of that particular geometry.

Interestingly, chelicerate embryos might also have an anterior bend. The work of Damen et al. (1998) shows, in a spider, a reduced pre-cheliceral En stripe apparently oriented perpendicular to all subsequent segmental stripes. A bend is also indicated by anterior mesoderm (Weygoldt 1985) and central nervous system (CNS) development (Wegerhoff and Breidbach 1985) in chelicerate embryos. They show that anterior mesoderm and brain structures shift dorsally and then caudally in chelicerates.

A labro-intercalary view of things

If we assume the existence of a labro-intercalary segment with an endite-only appendage, then a variety of puzzling inconsistencies are resolved:

Gene expression

The failure of ectopic *Antp* expression to affect the labrum might be explained by the labrum's endite nature. In the antennal segment, ectopic *Antp* transforms the antenna (telopodite) to thoracic leg (telopodite), and a comparable transformation would be expected in the labrum if it were a complete appendage. However, a labrum that consists of endites, but no telopodites, would not be transformed.

In the segment-specific stage of gene expression (the stage most highly conserved among insects; Rogers and Kaufman 1997), homeotic complex (HOX) genes are generally expressed in a colinear and contiguous pattern. Gap genes also control blocks of contiguous segments. However, the pregnathal segments do not appear to follow this pattern. In the traditional linear model (Fig. 2A), expression is discontinuous between the intercalary segment and the labrum, and also between the acron and the antenna. The S-model (Fig. 2B) eliminates expression gaps between the acron and antennal segments, but extends the gaps between the labrum and the intercalary segment. The L-/Bent-Y model (Fig. 2C) eliminates all expression gaps present in both the linear and S-models, except for the *Dll* mandibular expression gap common to all three models.
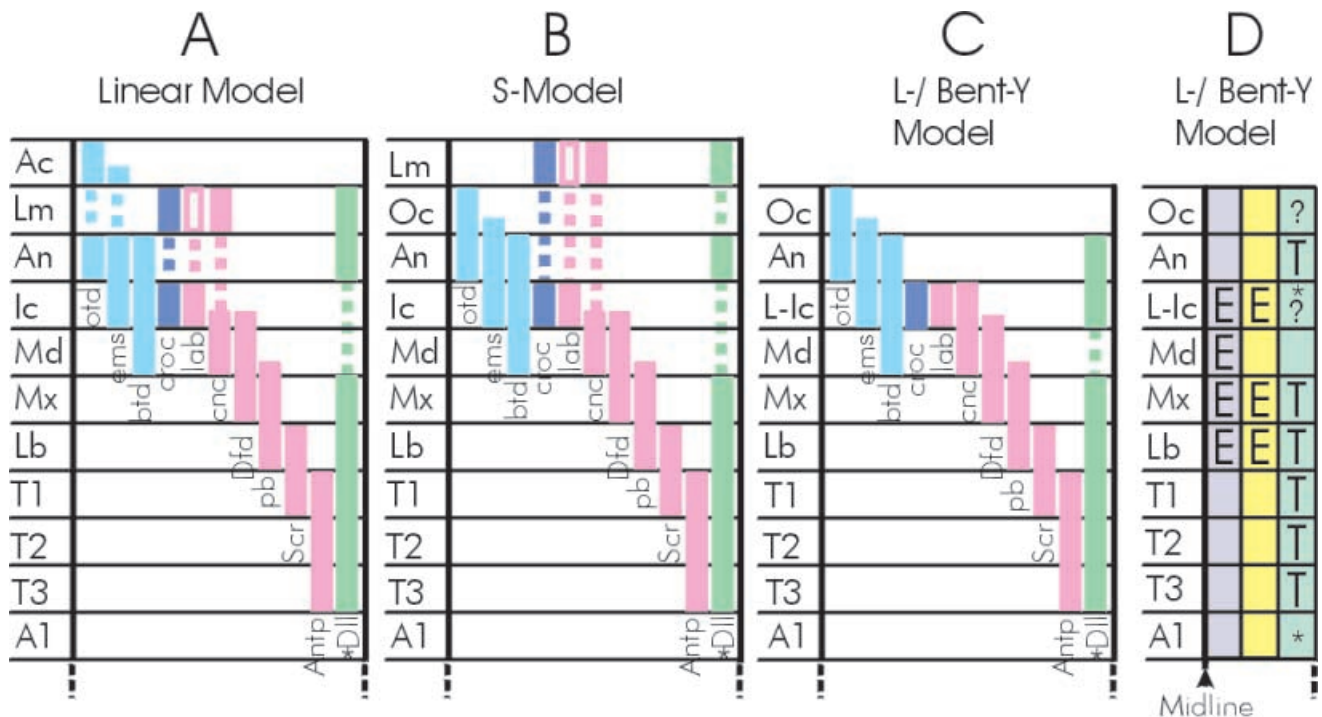
Fig. 2A–D Gene expression patterns and appendage composition. **A–C** *Drosophila* cuticular gene expression patterns during the segment-specific stage in three head segmentation models. **D** Adult appendage composition in *Tribolium castaneum*, L-/Bent-Y model. * Transient embryo appendage, *?* speculated, *Ac* acron, *An* antennal, *Ic* intercalary, *L-Ic* labro-intercalary, *Lb* labial (segment), *Lm* labral, *Md* mandibular, *Mx* maxillary, *Oc* ocular segment or ocular component of acron, *T1-T3* thoracic segments 1–3. Color code (**A–C**): *pink line* homeotic gene, *pink open line* no gene expression in *Drosophila* but expressed in crustacean *Porcellio scaber* (Abzhanov and Kaufman 1999), *light blue line* homeotic-like gap gene, *dark blue line* gap gene, *green line* appendage marker gene, *dotted lines* no gene expression. Color code (**D**): *lavender background* [endite (E), lacinia or homolog], *yellow background* [endite (E), galea or homolog], *light green background* [telopodite (T), palp or homolog]. Gene expression data sources as follows: *btd* (*buttonhead;* Cohen and Jürgens 1990), *cnc* (*cap'n'collar*; Mohler 1993; Rogers and Kaufman 1997), *croc* (*crocodile*; Hacker et al. 1995), *Dfd* (*Deformed*; Chadwick and McGinnis 1987), *Dll* (*Distal-less*; Cohen 1990), *ems* (*empty spiracles*; Cohen and Jürgens 1990), *lab* (*labial*; Diederich et al. 1989), *otd* (*orthodenticle*; Finkelstein and Perrimon 1990), *pb* (*proboscipedia*; Pultz et al. 1988), *Scr* (*Sex combs reduced*; Kuroiwa et al. 1985)

It should be noted that *cap'n'collar* (*cnc*) expression in the earlier blastoderm stage does show a distinct expression gap between the clypeolabral expression and the intercalary/mandibular expression in stained *Drosophila* embryos (Mohler 1993). However, during segment-specific development, the gap narrows considerably and nearly disappears. At stage 10, the mandibular expression extends anteriorly to the stomodeum, and at stage 12, the labral expression extends ventrally through the dorsal pharyngeal *hedgehog (hh)* domain. Similarly, in the milkweed bug, *cnc* expression is shown to occur at "the junction of" the fused clypeolabral and intercalary segments (Rogers and Kaufman 1997), "producing a ring of *cnc* expression". It is possible that the blastoderm expression gap in *Drosophila*

may be an artifact of the highly derived long germ band development in this species. Future studies of *cnc* expression in a wider variety of insects will help determine if this particular gap removal is an accurate reflection of common embryo morphology, or an artifact of the L-/Bent-Y model.

Innervation

Tritocerebral (intercalary) innervation of the insect labrum has been acknowledged uneasily and rationalized as a secondary derived condition (Snodgrass 1928). Snodgrass (1960) maintained that the tritocerebral innervation of the labrum is only sensory, and that the "motor innervation of the labral and clypeal muscles comes from the frontal ganglion". Alternatively, Rempel (1975) attributed the main innervation of the labrum to the recurrent nerve. By definition, primary innervation of segmental structures comes from the CNS. Both the unpaired recurrent nerve and the frontal ganglion are components of the insect stomatogastric nervous system (SNS) which is derived from foregut tissue and is distinctly separate from the CNS (Hartenstein 1997). Thus, neither the frontal ganglion nor the recurrent nerve can be considered a primary (CNS) motor innervation of a segmental appendage. Contrary to earlier assertions, primary motor innervation of the labrum by the tritocerebrum has been well documented in crustaceans (Robertson and Laverack 1979) and also noted in insects (Steinman 1970).

Embryogenesis

In a detailed histological study of embryogenesis in the beetle *Tenebrio molitor*, Ullmann (1964) observed that

both the labral and intercalary coelomic sacs exhibit several atypical characteristics compared to the other coelomic sacs. Each, by itself, is incomplete. Ullmann notes that "the labral sacs differ from the typical coelomic sacs in their almost spherical shape, eventual median location and histological uniformity" (cubical epithelium). They appear to "differentiate into splanchnic and somatic portions" but "do not form cardioblasts or fat body". She also notes that "the fate of the intercalary sacs is noteworthy" in that all other sacs "contribute to the somatic and splanchnic musculature." (Those of the intercalary do not.) These sacs are located closer to the midline than all other sacs except for those of the labrum. The intercalary sacs are also unique in that they appear after most other sacs have formed, and thus have a briefer existence than the others due to their delayed appearance. If these two apparently separate pairs of coelomic sacs are viewed as a continuum (Fig. 1F), irregularities in histology and timing of appearance are reconciled. Interestingly, the mesoderm of each spherical labral sac is continuous with a strand of mesoderm that trails back to the intercalary sac mesoderm located immediately behind the stomodeum. Thus, the intercalary sacs are in line and continuous with the labral coelomic sacs. [A similar alignment of labral and intercalary sacs was also noted by Rempel and Church (1969) in the beetle *Lytta viridana*.] The extension of the labral sacs toward the posterior is not recognizable as a sac extension as they circumvent the stomodeum, appearing there as merely a "stream" of mesoderm. Once posterior to the stomodeum, they again take on characteristics of sacs and complete their elongation, and are now recognized as the intercalary sacs. It is interesting to note that no mention is made of labral sac mesoderm being continuous posteriorly with either antennal or preantennal mesoderm as might be expected if the labrum was located immediately anterior to either of these two segments.

There are a number of similarities between the composite labro-intercalary coelomic sacs and the antennal coelomic sacs. Ullmann (1964) describes the antennal sacs as consisting of an appendiculate region, which descends into the developing antennal appendages, and the anal diverticula, which extend to the posterior, parallel to the body axis. This appendiculate region consists only of cubical epithelium, as also do the labral sacs, which might be homologous to this region of the antennal sacs. The intercalary sacs, when fully extended, resemble the antennal sac anal diverticula both in their orientation parallel to the body axis, and in their differentiation into columnar epithelium. Interestingly, the orientation of this portion of the antennal and labro-intercalary coelomic sacs is nearly perpendicular to that of the mesoderm of the "dorso-ventrally oriented" mandibular sacs. This geometry suggests a dorsal bend of these two pregnathal segments, as do *en* and *wg* expression patterns discussed above.

The stomodeum might have a repressing effect on the development of the labro-intercalary sacs, which are more closely associated with the stomodeum than are other sacs. It would be interesting to follow the development of the labral coelomic sacs in an insect mutant in which the stomodeum did not develop in order to determine if the intervening strands of mesoderm develop as sacs instead.

The eccentricities of labral and intercalary coelomic sacs complement each other. When viewed as the composite sacs of the labro-intercalary segment, they have all the attributes of the other coelomic sacs; they contribute to somatic and splanchnic musculature and to the cephalic aorta, and they no longer each have histological uniformity. In other words, together, they become a very ordinary pair of coelomic sacs, except for the fact that they lose their identity as sacs through that part of their length that is in close association with the stomodeum.

L-/Bent-Y model perspectives

It is possible that some of the appendage gene expression eccentricities traditionally attributed to the pregnathal segments might actually be artifacts of a misunderstood and "misplaced" segmental appendage – the labrum. Interpretations of gene expression pattern that are based on a misunderstanding of head segment order and composition could produce erroneous conclusions regarding gene function. For example, in contrast to recessive loss-of-function HOX genes, the hypomorphic loss-of-function mutation of the homeobox-containing master control gene *eyeless* (*ey*; Halder et al. 1995) causes a loss of eye structures. It would be expected that a loss-of-function mutation would transform the eye to an anterior (possibly labral, if it is first) segmental identity. However, if no segments exist anterior to the ocular, this lack of anteriorward transformation is predictable.

The assumption that the labrum is an endite of a combined labro-intercalary segment also invites speculations on possible commonalties of morphology and gene regulation between this segment and its neighboring mandibular segment. For example, in Haas et al. (in press) we propose a labrum composed solely of endites (enlarged "galeal" endites and reduced or vestigial "lacinial" endites; Fig. 2D). Thus, both labrum and mandible could be viewed as appendages having enlarged endites and repressed telopodites, perhaps due to shared *cnc, buttonhead* (*btd*), or *collier* (*col*; Crozatier et al. 1999) expression.

L-/Bent-Y model limitation

Though generally comprehensive, this model does not provide ready explanations for every controversy regarding the insect head capsule. One observation of uncertain significance involves the phenotype of buttonhead (*btd*) mutants in *Drosophila*. While *btd* is not expressed in the labrum, in *btd* mutants the antennal, intercalary and mandibular segment anlagen are missing, while the anlage of the labrum is retained (Cohen and Jürgens 1990). The

L-/Bent-Y model would predict that the labrum should follow the fate of the intercalary segment, but it does not. If this phenomenon is common among insects, rather than unique to the highly derived *Drosophila*, then it presents a possible contradiction to the L-/Bent-Y model.

Orthologies reconsidered

In summary, a diverse body of evidence exists that supports the view that the insect labrum is the appendage endite of the labro-intercalary segment. The L-/Bent-Y Model presented here provides cohesive explanations for many observed morphological, embryological, and molecular patterns. Thus, it promises to be a useful tool in the ongoing effort to decipher the nature of the procephalon. Continued molecular analysis will identify the valid components of existing models, and improve our understanding of the procephalon as well as clarify arthropod appendage orthologies.

The labra of chelicerates, crustaceans and insects express *Dll* (Popadic et al.1998). If the labrum is orthologous in these three arthropod classes, it would serve as a useful common reference point in resolving anterior segmental orthologies of the enigmatic chelicerate prosoma. In crustaceans and insects, the labrum receives primary motor innervation from the tritocerebral ganglion. If the less studied cheliceral ganglion (the presumed tritocerebral ortholog) is found to provide primary motor innervation to the labrum, a cheliceral-second antennal-intercalary segment orthology would be inferred. A more anterior origin of labral innervation would suggest a cheliceral-mandibular segment orthology. Manton (1960) refers to several works in which there is "precheliceral or cephalic coelom" associated with the labrum. This observation supports the latter possibility.

The composition of the chelicerate brain remains controversial. The deutocerebrum and its segment, perceived by some as absent (Damen et al. 1998; Telford and Thomas 1998), might exist unrecognizably fused with the protocerebrum (Weygoldt 1985). It is conceivable that, in contrast to the crustacean head, which bears two pairs of antennae, the chelicerate head might bear two pairs of eyes as the result of an antennal-to-ocular homeotic transformation that could have occurred at the origin of the chelicerate lineage.

The common ancestor of these arthropod classes probably had three pregnathal segments anterior to the stomodeum. Trilobites of the Cambrian era already had a defined pregnathal head consisting of eyes, antennae and a labrum, with subsequent segments relatively unspecialized (Manton 1977). The migration of the first three segments to a pre-oral position probably occurred in the Pre-Cambrian era (Snodgrass 1928). Onychophorans might represent an early point in this evolutionary process. In this organism, eyes and antennae are preoral, followed by weak jaw-like structures which are innervated by the first post-oral neuromere, the probable ortholog of the tritocerebrum. These jaws have been usually interpreted as mandibular orthologs, although Butt (1960) thought them to be tritocerebral structures and therefore labral orthologs. It will be interesting to see what continuing research on this organism will reveal about pregnathal evolution and orthology.

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